THE EFFECT OF LOCAL ANAESTHETICS ON STROPHANTHIDIN TOXICITY IN CANINE CARDIAC PURKINJE FIBRES

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SUMMARY

1. Canine Purkinje fibres were superfused *in vitro* and the electrical and mechanical effects of the local anaesthetics benzocaine and procaine were studied in the absence and in the presence of other agents.

2. Both benzocaine (1×10^{-4}–5×10^{-4} M) and procaine (6×10^{-5}–$2{\cdot}5 \times 10^{-4}$ M) decreased slightly the amplitude of the upstroke and markedly the duration of the action potential. The plateau was shifted to more negative values and the force of contraction was decreased. These effects were dose-dependent.

3. The local anaesthetics abolished the spontaneous activity induced by strophanthidin (5×10^{-7} M) by flattening the oscillatory potential in diastole and increased the force of contraction under these circumstances.

4. The local anaesthetics significantly delayed the time of the onset of the spontaneous activity induced by strophanthidin (10^{-6} M). Also, the intensity of the stimuli had to be increased and the rate of discharge of intoxicated fibres was slower in the presence of local anaesthetics. In contrast, the positive inotropic effect was little affected.

5. The local anaesthetics reduced but did not block the inotropic action of norepinephrine and high Ca; and did not abolish (while Mn did) small action potential in 27 mM-K-depolarized fibres.

6. In fibres treated with local anaesthetics, lowering $[Ca]_0$ did not result in a force rebound and administration of caffeine or exposure to low Na resulted in a larger increase in force.

7. In fibres loaded with Ca, local anaesthetics caused an increase in force.

8. Local anaesthetics decreased the force more when the external Na concentration was lower.

9. This work shows that the local anaesthetics alter the mechanical performance of Purkinje fibres and lead to a depression of the strophanthidin induced oscillatory potential. As for the mechanism for these changes, the present experiments support the hypothesis that local anaesthetic agents have an antiarrhythmic action by decreasing intracellular Na and therefore intracellular Ca as suggested by Perry, McKinney & DeWeer (1978) on the basis of experiments on nerve.

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Local anaesthetics are effective in the treatment of several arrhythmias (see Bassett & Hoffman, 1971), including those caused by cardiac glycosides (see Moe & Farah, 1975). The mechanism of this antiarrhythmic action is not entirely clear. Weidmann (1955) has shown that local anaesthetics reduce the maximal rate of rise and amplitude of the upstroke of the Purkinje fibre action potential, and shift the relation between the maximal rate of rise and the 'take-off' potential in a hyperpolarizing direction. Weidmann concluded that local anaesthetics inactivate the 'sodium carrier system' and this inactivation can be partially overcome by hyperpolarization. These findings have been confirmed since and extended to other local anaesthetics (see Bassett & Hoffman, 1971).

Because of the effect of local anaesthetics on the fast Na conductance, a number of effects are brought about (diminished conduction velocity, reduced excitability and increased refractory period) which are important in accounting for the antiarrhythmic action of these agents. Whether other actions might also be important is quite conceivable, for cardiac glycosides often induce spontaneous activity in Purkinje fibres by means of transient depolarization superimposed on diastolic depolarization (see Ferrier, 1977). The transient depolarizations are sensitive to extracellular Ca concentration and to agents which modify Ca movements across the cell membrane (Ferrier & Moe, 1973) and are caused by an increase in intracellular Ca concentration (Kass, Lederer, Tsien & Weingart, 1978a; Kass, Tsien & Weingart, 1978b). However, the ionic mechanism underlying the transient depolarizations appear also to involve Na since this ion has been shown to be important in the induction of spontaneous activity by cardiac glycosides (Vassalle, 1975; Lin & Vassalle, 1978; Vassalle & Scida, 1979; Rosen & Denilo, 1980) and in current underlying transient oscillations (Kass *et al.* 1978b).

These findings elicit the question as to whether local anaesthetics might counteract digitalis arrhythmias not only by reducing the fast inward current but also by specifically affecting the transient depolarizations. In fact, the amplitude of digitalis-induced transient depolarizations is reduced by diphenylhydrantoin (Rosen, Danilo, Alonso & Pippenger, 1976) and by aprindine (Elharrar, Bailey, Lathrop & Zipes, 1977) while the transient inward current induced by low $[K]_o$ is abolished by lidocaine (Eisner & Lederer, 1979). Local anaesthetics could affect transient depolarizations also by reducing cellular Ca as it has been shown in other tissues that local anaesthetics reduce the force of contraction (see Bianchi & Strobel, 1968) and tetracaine has been reported to abolish the slow inward current (Eisner, Lederer & Noble, 1979). Since both Ca and Na are involved (in different ways) in the induction of transient depolarizations, it is conceivable that an antiarrhythmic action of local anaesthetics may involve interference with either ion (or both). The aims of the present experiments were to test whether the local anaesthetics procaine and benzocaine counteract strophanthidin toxicity in cardiac Purkinje fibres perfused *in vitro* and whether such an action involves an effect on transient depolarizations. Simultaneous recording of electrical and mechanical activity suggests that the antiarrhythmic action of these local anaesthetics involves an interference with Na movements not only during the upstroke but also during the action potential and

that a reduced Na inward movement may result in a fall in $[Na]_i$ and therefore in $[Ca]_i$, as proposed by Perry, *et al.* (1978) on the basis of their experiments on nerve.

The present results have been reported in abstract form (Bhattacharyya & Vassalle, 1979).

METHODS

Mongrel dogs of either sex (12–22 kg) were anaesthesized with Na pentobarbitone (30 mg/kg I.V.). The heart was excised through an intercostal incision. Strands of Purkinje fibres (0·5–1 mm in diameter) from the right or left ventricle were superfused in a tissue bath with oxygenated (97 % O_2 and 3 % CO_2) Tyrode solution at 37 °C. One end of the Purkinje strand was kept in place by means of a steel pin insulated electrically except for the tip. Another steel pin was located near the strand. The pins were connected to a Grass Stimulator (Model S4) via a Grass Stimulation Isolation Unit (Model SIU 4678). The rate of stimulation was usually 60/min, the duration of the pulses 1·5–2 msec and the voltage 50 % higher than the threshold. The composition of the Tyrode solution was as follows (mM): NaCl 137; KCl 2·7; NaHCO$_3$ 11·9; NaH$_2$PO$_4$ 0·45; MgCl$_2$ 0·5; CaCl$_2$ 2·7; dextrose 5·5.

The transmembrane potentials were recorded by means of two glass micro-electrodes filled with 3 M-KCl and connected to a cathode follower stage. The tip of one micro-electrode was inserted into a cell and that of the other was immersed in perfusion fluid. The potential was displayed on a Tektronix model 502A dual beam oscilloscope. The mechanical activity of the Purkinje fibres was recorded simultaneously with the electrical activity. One end of the fibre was fixed with the rigid stainless-steel pin used also as stimulating electrode and the other was connected by means of a short silk thread to the end of the steel rod attached to a force displacement transducer (Grass, Model FTO 3C).

With the preparation driven at 60/min in normal Tyrode solution, the length of the fibre was increased in steps until maximal contractile force was developed. The length was then decreased again to a value which resulted in 60 % of the maximal contractile force. A Grass Model 7 polygraph was used to record force at a paper speed of 0·25 mm/sec. The force curve was also displayed on the Tektronix oscilloscope. The electrical and mechanical events displayed in the oscilloscope were photographed with a Grass Kymograph Camera (Model C4C). At the beginning of the experiment, the preparations were allowed to equilibrate in normal Tyrode solution for about an hour. The stock solution of strophanthidin (Sigma Chemical Co.) was prepared the day before the experiment and was stored in the refrigerator. The solution of norepinephrine (Levophed bitartrate. Winthrop Laboratories) was prepared during the experiment. After an exposure to strophanthidin, the fibres were allowed to recover fully. The local anaesthetics benzocaine (ethyl-*p*-aminobenzoate, Sigma Chemical Co.) and procaine (procaine hydrochloride. Sigma Chemical Co.) were freshly dissolved in Tyrode solution on the day of the experiment. Changes in action potential were measured under magnification. The amplitude of the upstroke was measured from traces recorded at different speeds.

Statistical analysis of the data included the determination of average values ± standard error (S.E.) and the Student's t test: a P value less than 0·05 was taken to indicate a statistically significant difference.

RESULTS

The effects of benzocaine and procaine on electrical and mechanical activity

The effects of benzocaine and procaine on the action potential and contractile force are illustrated in Fig. 1. It is apparent that both agents shifted the plateau to a more negative value, shortened the duration of the action potential (traces identified by the arrows) and abolished the force development. In traces recorded at a faster time base (not shown), benzocaine was found to have little effect on the rapid repolarization, while procaine shifted the end of rapid repolarization to a more positive value. When four different concentrations of benzocaine (5×10^{-4}–$7·7 \times 10^{-6}$ M) and of procaine ($2·5 \times 10^{-4}$–$3·9 \times 10^{-6}$ M) were tested, it was found that the effects illustrated in Fig. 1 were dose-dependent, occurred sooner the higher the concentration and were fully reversible at all concentrations.

In eleven experiments, benzocaine (1.25×10^{-4} M) decreased the amplitude of the upstroke by $7.1 \pm 1.1\%$ and the duration of the action potential (measured at 50% of its amplitude) from a control value of 291.9 ± 16.9 to 146.8 ± 12.7 msec (-50%, $P < 0.001$). Benzocaine increased slightly the slope of diastolic depolarization from 6.6 ± 0.9 mV/sec to 9.6 ± 1.1 mV/sec ($P < 0.02$, $n = 9$). Twitch force was decreased by $85.5 \pm 4.5\%$.
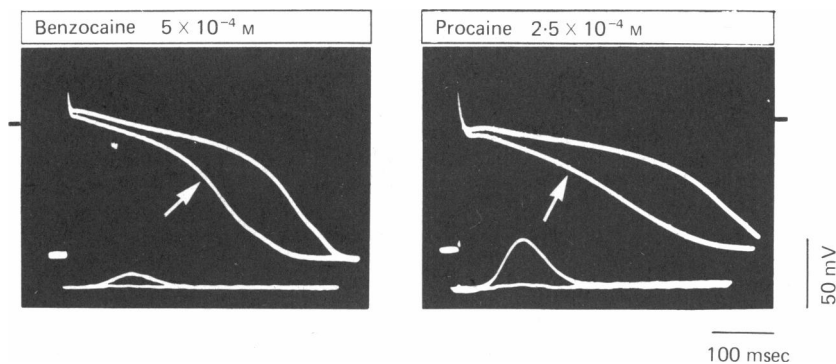

Fig. 1. The effect of benzocaine and procaine on the electrical and mechanical events in Purkinje fibres. In each panel, two action potentials are shown: one in the absence and another (labelled with an arrow) in the presence of the local anaesthetic. The two panels were taken from different experiments but in each panel the action potentials were recorded from the same fibre. In each panel, the smaller twitch was recorded in the presence of the local anaesthetic. Voltage and time calibrations are shown at the lower right hand corner of the picture. In this and the following Figures, the zero potential is indicated by the small horizontal bar next to the traces.

In eight experiments, procaine (2.5×10^{-4} M) decreased the amplitude of the upstroke by $5.7 \pm 0.7\%$ and the duration of the action potential (at the 50% level) from 320.7 ± 19.7 to 177.6 ± 10.4 msec (-45%, $P < 0.001$). In these experiments, procaine did not modify diastolic depolarization (6.8 ± 1.3 mV/sec in the control and 7.4 ± 1.5 mV/sec in the presence of procaine). Twitch force was decreased by $79.0 \pm 4.6\%$.

Effect of strophanthidin in the absence and presence of benzocaine

Strophanthidin causes a progressive increase and then a gradual decrease in force of contraction in cardiac Purkinje fibres (Lin & Vassalle, 1978; Vassalle & Lin, 1979). It is when the force declines that usually spontaneous activity occurs (Lin & Vassalle, 1978). In Fig. 2, the top strip shows the action potentials recorded at the time indicated by the respective dots above the slow speed mechanical record (bottom strip). The increase in force induced by strophanthidin was already declining when the steepened diastolic depolarization induced spontaneous activity intermingled with driven action potentials (second top panel). When this occurred, benzocaine was added to the superfusing solution and it is apparent that within 2 min, the spontaneous activity disappeared, the diastolic depolarization became flatter (third top panel) and the force of contraction increased (second bottom panel). When benzocaine superfusion was discontinued, the transient oscillation became more pronounced (fourth top panel) and eventually irregular activity ensued again.

In five experiments, strophanthidin (5×10^{-7} M) alone increased the twitch force by $180.8 \pm 47.0\%$ and caused the usual patterns of intoxication; and benzocaine (2.5×10^{-4} M) alone decreased the twitch force by $77.5 \pm 4.4\%$. When benzocaine was given during strophanthidin-induced spontaneous activity, consistently the spontaneous activity was suppressed and the transient oscillations were reduced in amplitude. Similar results were obtained with procaine (2.5×10^{-4} M, three experiments). In five of these eight experiments, the force of contraction increased by $31.2 \pm 10.5\%$ ($P < 0.01$) when local anaesthetics were given during the declining phase of strophanthidin inotropy.
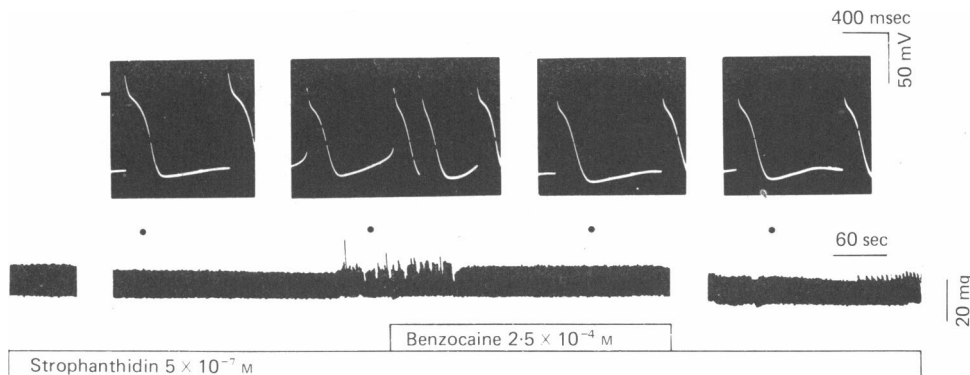

Fig. 2. Abolition of strophanthidin-induced spontaneous activity in the presence of strophanthidin. The top strip shows the action potentials during the declining phase of strophanthidin inotropy (first panel), during the spontaneous activity (second panel), during the addition of benzocaine (third panel) and again in the presence of strophanthidin alone (fourth panel). The bottom strip shows mechanical events at slow speed during the peak inotropic effect of strophanthidin (first panel), during the simultaneous superfusion of strophanthidin and benzocaine (second panel) and during strophanthidin alone (third panel). Time, voltage and force calibration are on the right side of the figure. In the bottom strip the interruption in the traces are 6 min 20 sec and 5 min 10 sec, respectively.
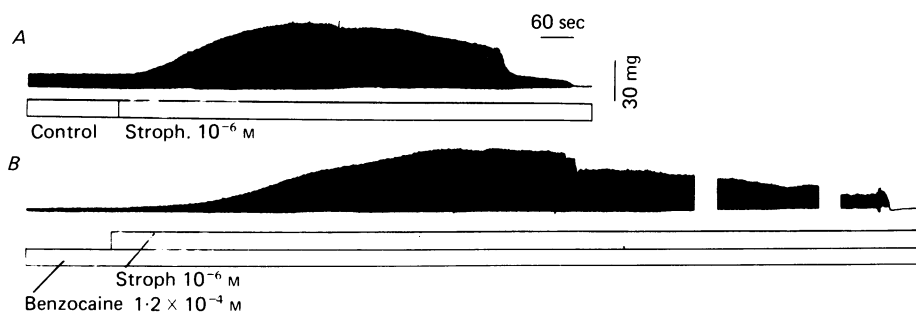

Fig. 3. The inotropic effect of strophanthidin in the absence and presence of benzocaine. In strip A, the slow speed record begins with the contractile force in Tyrode solution. Strophanthidin was added as indicated below the trace. Spontaneous activity began shortly after the maximal force was attained. In strip B, benzocaine was superfused throughout and strophanthidin added as indicated. Spontaneous activity occurred at the sudden decrease in force in the first panel. The traces in A and B were recorded from the same preparation. Time and force calibrations apply to both strips. The interruptions in the traces in strip B were 2 min 30 sec and 5 min and 5 sec, respectively.

In another approach, the preparations were first exposed to strophanthidin until toxicity occurred. After full recovery, the fibres were superfused with benzocaine alone for 20 min. Finally, benzocaine was given for 5 min and strophanthidin was then added. In Fig. 3*A*, the panel shows that strophanthidin increased and then decreased the twitch force until spontaneous activity developed after 390 sec. In *B*, benzocaine had decreased the twitch force by 71 % at the beginning of the first panel. When strophanthidin was added, it induced the usual positive inotropic effect which, however, developed much more slowly. Spontaneous activity occurred after 855 sec of strophanthidin exposure and the spontaneous rhythm lasted much longer than in the absence of benzocaine. Electrical recordings (not shown) made clear that diastolic depolarization was flatter and the spontaneous activity slower (61/min *vs.* 122/min) when strophanthidin was administered in the presence of benzocaine. In the presence of both drugs, the stimulus intensity had to be increased.

In ten experiments, strophanthidin induced spontaneous activity in 292.5 ± 56 sec in the absence and in 823.1 ± 123.5 sec $(+181.4\%, P < 0.005)$ in the presence of benzocaine. In the presence of benzocaine, the time to maximal twitch force was longer $(668 \pm 142$ sec, $+275\%, P < 0.005)$, the maximal twitch force less $(-37\%, P < 0.05)$ and the rate of spontaneous activity slower (control 121.3 ± 6.5/min, benzocaine 61.1 ± 5.3/min, $-49\%, P < 0.001)$. However, the increment in force was larger in the presence of benzocaine $(+66 \pm 13\%, P < 0.005)$.

The results with procaine (tested according to the same protocol) were similar. In eight experiments, strophanthidin induced spontaneous activity in 221.2 ± 10.7 sec in the absence and 1150 ± 131.1 sec $(+419\%, P < 0.001)$ in the presence of procaine. In the presence of procaine, the time to maximal twitch force was longer $(856.6 \pm 75.9$ sec, $+449\%, P < 0.001)$, the maximal twitch force greater $(15\%, P < 0.05)$ and the rate of the spontaneous activity slower (control 122.9 ± 5.3/min, procaine 51.8 ± 3.4/min, $-58\%, P < 0.001)$. The spontaneous rhythm was actually slower than the driving rate: when spontaneous activity appeared the drive was discontinued and the spontaneous activity settled at the mean value indicated.

Effects of norepinephrine in the presence of local anaesthetics

The results reported so far show that benzocaine and procaine counteract strophanthidin-induced spontaneous activity by depressing the transient oscillation during diastole. The effect could result from a block of Na channels and therefore a decrease in $[\text{Na}]_i$ (see Perry *et al.* 1978) but an additional effect on Ca influx cannot be excluded. If benzocaine and procaine decrease the force of contraction by hindering the inward Ca movement through the slow channel, then local anaesthetics may impair the increase in force by agents known to increase the slow inward current.

In Fig. 4, benzocaine had the usual effects (compare the first and the second panel). Norepinephrine shifted the beginning of the plateau to a more positive value (arrow in the third panel recorded at a higher speed) and also increased the contractile force above the pre-benzocaine control (fourth panel). Norepinephrine increased the slope of diastolic depolarization (not shown) and increased the maximum diastolic potential (fourth panel). Similar changes were observed in the presence of procaine.

In eight experiments, norepinephrine increased the force to a maximum of 41.9 ± 10.9 mg in the absence and 24.3 ± 8.1 mg $(-42\%, P < 0.05)$ in the presence of benzocaine. The time to maximal twitch force development was 156.2 ± 22.0 sec in

the absence and $223 \cdot 5 \pm 29 \cdot 0$ sec $(P < 0 \cdot 01)$ in the presence of benzocaine. In six experiments, norepinephrine increased the force of contraction to a maximum of $27 \cdot 5 \pm 8 \cdot 6$ mg in the absence and $11 \cdot 7 \pm 2 \cdot 5$ mg in the presence of procaine $(P < 0 \cdot 01)$. The time to maximal twitch force development was $158 \cdot 0 \pm 36 \cdot 0$ sec in the absence and $197 \cdot 0 \pm 33 \cdot 0$ sec in the presence of procaine $(P > 0 \cdot 1)$.
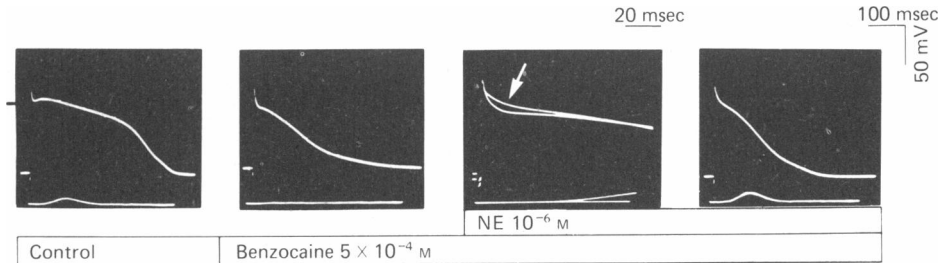

Fig. 4. Effect of norepinephrine on electrical and mechanical events in the presence of benzocaine. The first panel shows the control, the second the changes in the presence of benzocaine, the third the superimposed traces showing the effect of norepinephrine on initial part of the plateau (arrow) in the presence of benzocaine. The fourth panel shows the effect of norepinephrine in the presence of benzocaine on the action potential and the contractile force. Time calibrations for the third panel is above that panel and for the other panels is above the last one.

When the increment in force (rather than the absolute value) is considered, norepinephrine was almost as effective in the absence $(25 \cdot 4 \pm 7 \cdot 9$ mg) as in the presence $(21 \cdot 1 \pm 7 \cdot 1$ mg) of benzocaine $(P > 0 \cdot 1)$. For procaine, the difference was larger but also not statistically significant ($17 \cdot 9 \pm 6 \cdot 4$ mg and $10 \cdot 7 \pm 3 \cdot 6$ mg respectively, $P > 0 \cdot 1$).

Effects of Ca in the presence of local anaesthetics

Higher Ca was also tested for its inotropic effect. The effect of high Ca in the presence of procaine and the effect of procaine in the presence of high Ca are illustrated in Fig. 5. High Ca quickly increased the contractile force in the presence of procaine (first A panel), induced a marked notch, shortened the action potential and increased maximum diastolic potential (trace indicated by the arrows in the last A panel). When procaine was applied in the presence of high Ca, the force fell markedly (first B panel), the plateau shifted to more negative values and the maximum diastolic potential decreased (trace indicated by arrows in the last B panel). Similar results were obtained with benzocaine.

The force with benzocaine was $1 \cdot 8 \pm 1 \cdot 3$ mg and increased in high Ca to $28 \cdot 2 \pm 15 \cdot 1$ ($+ 1466 \%$, $n = 3$); the corresponding values for procaine were $1 \cdot 8 \pm 0 \cdot 7$ and $12 \cdot 8 \pm 6 \cdot 4$ ($+ 611 \%$, $n = 3$). The contractile force in high Ca was $50 \cdot 9 \pm 15 \cdot 7$ mg and benzocaine decreased it to $18 \cdot 1 \pm 10 \cdot 9$ mg ($- 64 \%$, $n = 3$); the corresponding values for procaine were $33 \cdot 2 \pm 14 \cdot 5$ mg and $8 \cdot 9 \pm 3 \cdot 3$ mg, respectively ($- 73 \%$, $n = 3$).

If the increment in force (rather than the absolute value) is considered, high Ca still was less effective in increasing force in the presence of benzocaine ($- 26 \cdot 5 \%$) and of procaine ($- 54 \cdot 1 \%$). This was a consistent finding, although the difference was not significant.
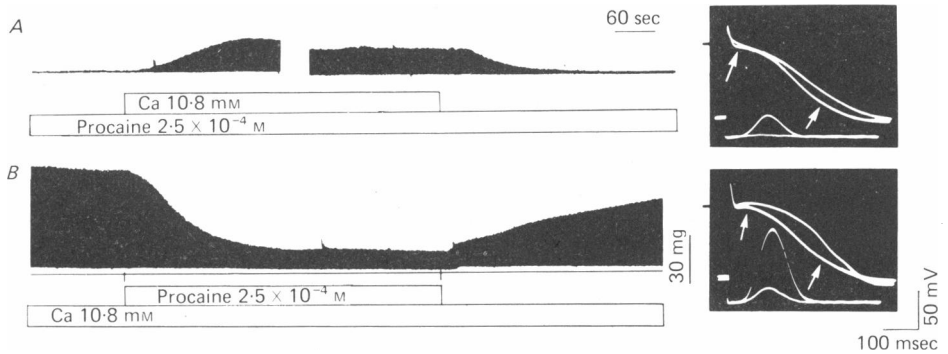
Fig. 5. Interactions between Ca and procaine. *A*, the first panel shows the effect of increasing Ca on force of contraction in the presence of procaine and the second panel the fall in force as $[Ca]_0$ was decreased. The last panel shows the superimposed recording of the action potential and force in the presence of procaine and of procaine and high Ca (arrows). The interruption between the first and the second panel was 2 min 30 sec. *B*, the first panel shows the force recording in high Ca and the fall in force induced by procaine in the presence of high Ca. The second panel shows the superimposed recording in the presence of high Ca and of high Ca and procaine (arrows). Both strips were recorded from the same preparation.

Local anaesthetics and low Ca

During a prolonged exposure to strophanthidin (Lin & Vassalle, 1978) or when $[Ca]_0$ is increased beyond 8 mM (Vassalle & Lin, 1979), the twitch force decreases. If external Ca is then decreased in either condition, there is a transient increase in force ('rebound phenomenon'). If the decrease in twitch force induced by local anaesthetic involves changes similar to those induced by the late strophanthidin exposure or high Ca, then a rebound phenomenon may occur also when external Ca is reduced in the presence of local anaesthetics. Otherwise, a decrease in external Ca should lead to the usual decrease in twitch force. Once the local anaesthetics had decreased the twitch force, $[Ca]_0$ was reduced to 0·54 mM: the twitch force declined to base line (without any rebound increase) while the action potential lengthened as it does in Tyrode solution. Similar results were obtained in four experiments with procaine and one experiment with benzocaine.

The effect of caffeine in the presence of local anaesthetics

Local anaesthetics could decrease force by decreasing internal Ca. To gain information on this point, caffeine was used. Caffeine has been shown to increase and then decrease twitch force in Purkinje fibres but to cause a sustained increase in twitch force when $[Ca]_0$ is low (Lin & Vassalle, 1979) and therefore $[Ca]_i$ is presumably also decreased. If local anaesthetics decrease $[Ca]_i$, caffeine should increase (but not decrease) force in their presence. In Fig. 6 (top strip) benzocaine caused the usual decline in force (first panel). When caffeine was added to the solution (middle panel), the force was greater as long as caffeine was present. The last panel shows the recovery in Tyrode solution. In Fig. 6 (bottom strip) procaine caused the usual fall in force (first panel) and caffein increased force as long as its perfusion lasted (middle panel).

In twelve experiments, caffeine initially increased ($+27 \pm 6 \cdot 7 \%$) and then decreased ($-33 \cdot 1 \pm 7 \cdot 6 \%$) contractile force (see Vassalle & Lin, 1979). When caffeine was administered in the presence of benzocaine ($n = 5$), the initial increase was $92 \cdot 8 \% \pm 17 \cdot 8 \%$ and the force remained $20 \pm 5 \cdot 5 \%$ above that in benzocaine alone. Similar results were obtained in the presence of procaine ($+113 \pm 35 \%$ and $+23 \cdot 5 \pm 8 \cdot 1 \%$, respectively, $n = 5$).
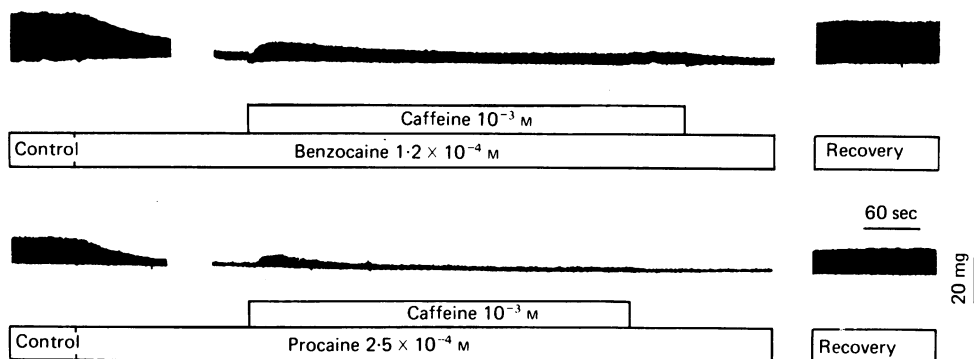

Fig. 6. The effect of caffeine in the presence of local anaesthetics. In the top strip, the first panel shows the decrease in force by benzocaine; the middle panel the effect of caffeine; and the last panel the recovery in Tyrode solution. The interruptions in the strip are 7 min 40 sec and 12 min 35 sec. In the bottom strip, the first panel shows the fall in force by procain in a different experiment. The second panel shows the sustained increase in force induced by adding caffeine. The last panel shows the recovery in Tyrode solution. The interruptions in the trace are 4 min 50 sec and 8 min 5 sec.

Caffeine in the presence of local anaesthetics and high Ca

While the results in the previous section suggest that the local anaesthetics cause a fall in $[Ca]_i$, local anaesthetics could also allow less Ca to be liberated from the sarcoplasmic reticulum (Almers & Best, 1976; Caputo, Vergara & Bezanilla, 1979). Since caffeine releases Ca from the sarcoplasmic reticulum (Thorpe, 1973; Kerrick & Best, 1974) in cardiac muscle cells, this point was tested in the manner illustrated in Fig. 7. In A, the usual effect of caffeine is seen (an increase followed by a subsequent decline in force). In B, high Ca and procaine were administered simultaneously. A comparison with the first panel in C (high Ca alone) and D (procaine alone) shows that the simultaneous administration of high Ca and procaine results in a much smaller increase in force than with high Ca alone. When caffeine was added in the presence of both high Ca and procaine, the force increased initially far more than in Tyrode solution and the increase was sustained. This result suggests that procaine did not prevent either the filling up of sarcoplasmic reticulum stores by high Ca or the release of Ca from these stores by caffeine. As shown in C, second panel, caffeine had a purely negative inotropic effect when applied in the presence of high Ca, as expected (Vassalle & Lin, 1979). As usually found, caffeine had little or no effect on the action potential configuration whether it was administered in Tyrode solution, in the presence of procaine, of high Ca or of both. Procaine had the usual effects on the action potential (e.g. Figs. 1 and 5). In seven experiments, caffeine alone caused an initial increase of $52 \cdot 1 \pm 10 \cdot 6 \%$ and a subsequent decrease of $23 \cdot 5 \pm 7 \cdot 1 \%$. High Ca

plus procaine induced a maximal increase in force of $256 \pm 103{\cdot}7\ \%$; when caffeine was added, the force rose above the pre-caffeine level by $106 \pm 17{\cdot}5\ \%$. In four experiments, caffeine alone caused an initial increase in force of $38{\cdot}7 \pm 9{\cdot}5\ \%$ and a subsequent decrease of $29{\cdot}0 \pm 9{\cdot}3\ \%$. High Ca plus benzocaine induced a maximal increase in force of $168{\cdot}0 \pm 84{\cdot}2\ \%$; when caffeine was added the force rose above the pre-caffeine level by $76{\cdot}0 \pm 18{\cdot}9\ \%$. Since the increase in force with caffeine in the presence of high Ca and local anaesthetics was larger than at lower $[Ca]_o$, the results are unlikely to be due to a caffeine-induced shift of force–$[Ca]_o$ relationship (see Fig. 9 of Vassalle & Lin, 1979) to the left.
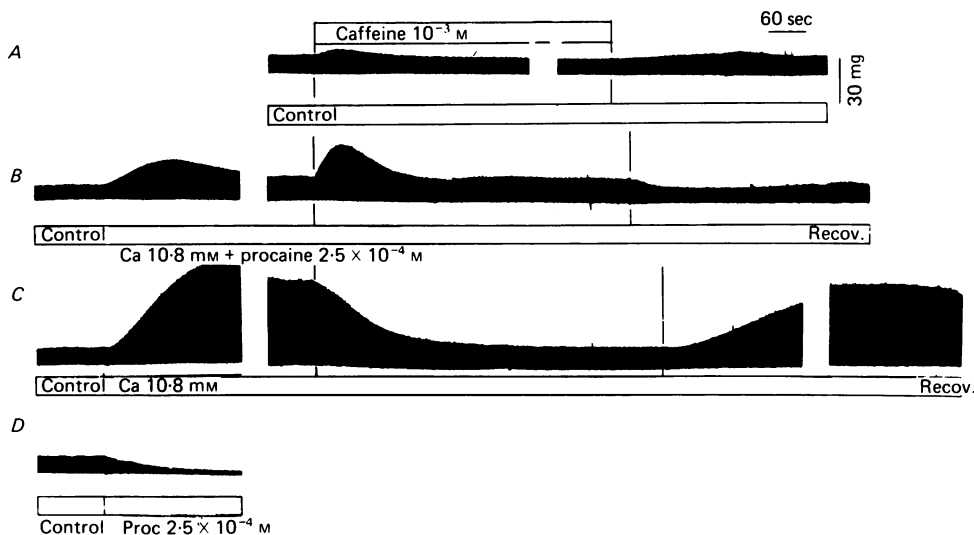

Fig. 7. The effect of caffeine in the presence of high Ca and procaine. A, the first panel shows the initial increase in contractile force and then subsequent decrease induced caffeine. The second panel shows the recovery. The interruption in the trace is 7 min 10 sec. B, the first panel shows the increase in force due to procaine and high Ca. The second panel shows the marked increase in force and then a subsequent decrease induced by caffeine. The interruption between the two panels is 5 min. C, the first panel shows the increase in force in high Ca. The middle panel shows the decrease in force as caffeine is added in the presence of high Ca and the subsequent rise in force as caffeine is withdrawn. The last panel shows the recovery in high Ca. The interruption between the first two panels is 4 min 30 sec, and that between the last two panels is 4 min 25 sec. D, the panel shows the decline in force due to procaine ($2{\cdot}5 \times 10^{-4}$ M) alone. All strips were recorded from the same preparation.

Increase in contractile force by local anaesthetics: the rebound phenomenon

If caffeine is more effective in increasing force in the presence of local anaesthetics and high calcium because local anaesthetics decrease Ca overload, then procaine may increase (and not decrease) the force when overload is present. This was tested by overloading the fibres in the presence of high ($8{\cdot}1$ mM) Ca and caffeine (1 mM). In Fig. 8, top strip, in Tyrode solution caffeine caused the initial increase in force followed by a decline (first panel) and procaine the usual decline in force ($-63\ \%$, second panel). In the bottom strip, first panel, high Ca had increased the force and caffeine decreased it. When procaine was added to the high Ca caffeine solution (second panel), the force

increased by 65 % (in contrast to the decline in Tyrode solution). The effects of procaine (second panel) and caffeine (third panel) were reversible. As usual, caffeine had little effect on the action potential configuration either in normal or high Ca. Procaine modified the action potential in the usual manner in the presence of caffeine alone and of caffeine and high Ca.
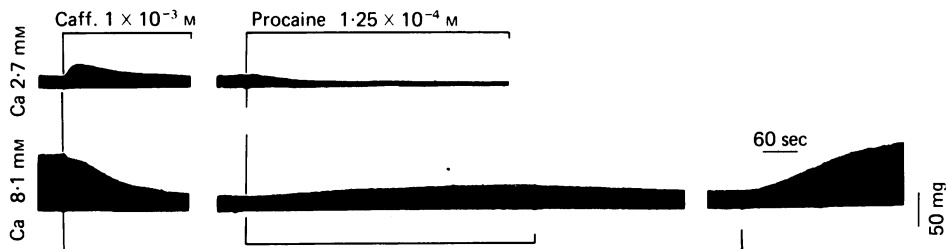

Fig. 8. Positive inotropic effect of procaine in the presence of calcium overload. In the top strip (Ca 2·7 mM) the effects of caffeine and procaine given separately are shown. In the bottom strip (Ca 8·1 mM) the first panel shows the increase in force induced by high Ca and the decline by caffeine. The middle panel shows the increment in force induced by pιocaine in the presence of high Ca and caffeine. The last panel shows the recovery in high Ca. In the bottom panel the interruptions are 2 min 35 sec and 1 min 5 sec, respectively. All traces are from the same preparation.
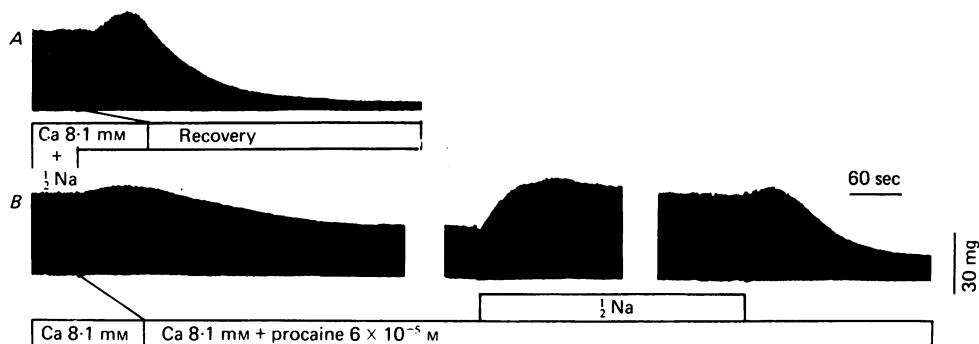

Fig. 9. *A*, the trace shows the mechanical activity in high Ca, low Na solution and the rebound increase in force during recovery in Tyrode solution. *B*, the first panel shows the mechanical activity in a high calcium solution and the initial rebound in force when procaine was added. The middle panel shows the increase in force induced by lowering the Na in the solution. The last panel shows the rebound increase in force during the recovery in Tyrode solution. All traces are from the same preparation.

In four experiments, procaine decreased the force of contraction by $67\cdot8\pm3\cdot1$ % in Tyrode solution and increased it by $71\cdot5\pm26\cdot9$ % in the presence of high Ca and caffeine ($P < 0\cdot02$). In one experiment, benzocaine decreased the force of contraction in both Tyrode (-70 %) and high Ca plus caffeine (-15 %) solutions, but obviously less in the latter.

In another approach, the fibres were Ca overloaded by using a high (8·1 mM) Ca, low (-50 %) sodium solution. As expected, under these conditions a rebound in force occurred during the recovery in Tyrode solution (Fig. 9A). When the same preparation was superfused with high Ca alone, procaine caused an initial rebound in force followed by the usual decline (strip *B*, first panel). When sodium concentration was

reduced by half in the presence of high Ca and procaine, force increased quickly to a value greater than control (see strip A). High Ca had the usual effects on action potential configuration. Lowering the external Na concentration decreased the action potential duration in the presence the high Ca and of high Ca and procaine. During the rebound increase in force, the action potential was modified by procaine in the usual way.

In six experiments, 8·1 mM Ca increased the force by $372·4 \pm 92·8\%$ from a control value of $8·8 \pm 0·9$ mg. Procaine caused a rebound increase in force in the presence of high Ca ($+10·8 \pm 3·6\%$, $P < 0·025$). In four of these experiments, low sodium in the presence of high Ca plus procaine increased force by $31·0 \pm 22·7\%$ above the control value in high Ca low Na solution without procaine. The force rebound in the control run when the low Na high Ca solution was changed to Tyrode solution was rather substantial ($+44·2 \pm 9·7\%$).

Internal Na and the negative inotropic effect of local anaesthetics

Local anaesthetics could decrease the force of contraction by reducing the Na influx and therefore $[Na]_i$. If this is the case, then the negative inotropic effects should become more marked when the Na influx is already reduced. To test this, $[Na]_o$ was reduced while maintaining the $[Ca]_o/[Na]_o^2$ constant (Lüttgau & Niedergerke, 1958). In actuality, small adjustments in Ca concentration with respect to the calculated value were made to maintain the force approximately constant. The osmolarity of the solution was kept unchanged by adding sucrose in appropriate amounts. In four experiments, the force in Tyrode and in the low Na low Ca solution was similar ($11·2 \pm 1·5$ *vs.* $11·8 \pm 1·7$ mg). Procaine decreased contractile force by $75·6 \pm 1·4\%$ in Tyrode and by $88·5 \pm 3·9\%$ ($P < 0·05$) in low Na low Ca solution. In a fifth experiment, benzocaine decreased the force by 59% in Tyrode and to 0 in low Na low Ca solution.

Local anaesthetics and slow response

The experiments with norepinephrine and high Ca suggest that local anaesthetics are unlikely to decrease contraction by blocking the slow channel. If this is so, then local anaesthetics should not eliminate the small action potentials due to the activation of the slow channel which are recorded in depolarized fibres (Carmeliet & Vereecke, 1969; Pappano, 1970). In the present experiments, the fibres were perfused in a solution containing 27 mM-K and 10^{-6} M-norepinephrine. The osmolarity of the solution was maintained by decreasing $[Na]_o$. The fibres were driven at 30/min. In Fig. 10, it is apparent that procaine did not affect the upstroke or the amplitude of the action potential: the only effect was a prolongation of the action potential (trace marked by an arrow in the second top panel). The first bottom panel shows the action potential before and the second panel the action potential during the exposure to the slow channel blocker Mn (1 mM): manganese gradually decreased the amplitude of the action potential and eventually abolished it.

These findings were consistently obtained in five experiments and indicate that the slow channel is not blocked by procaine. It must be pointed out that the force of contraction still decreased with either procaine or Mn. Therefore, either procaine reduced the internal Na concentration even at a depolarized level (see Deitmer & Ellis, 1980) or had some other effect on the excitation–contraction process.
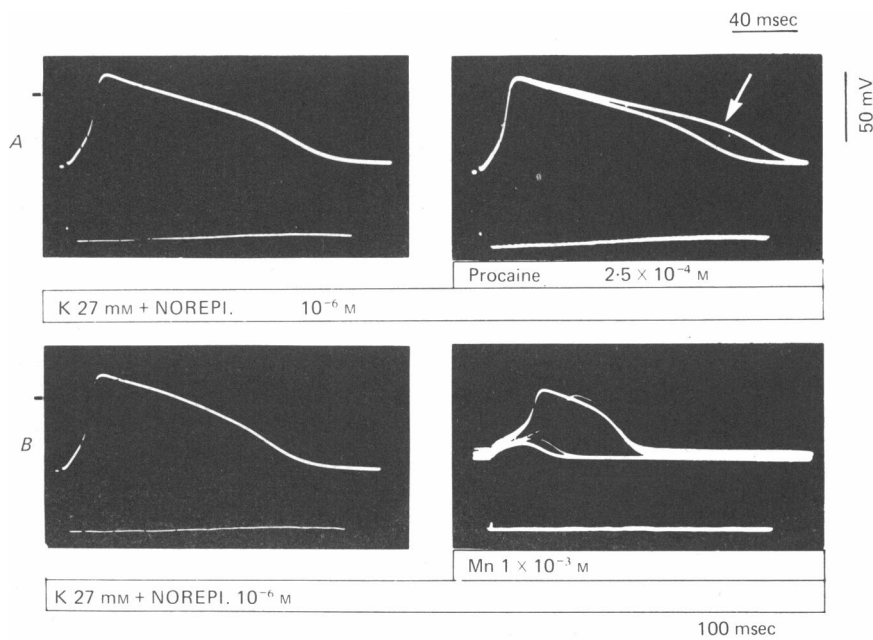
Fig. 10. Effect of procaine and Mn on the slow action potential response. *A*, the first panel shows the mechanical and electrical recording in high K and norepinephrine solution. The second panel shows the superimposed traces in the absence and presence (arrow) of procaine. *B*, the first panel shows the mechanical and electrical recordings in high K and norepinephrine solutions. The second panel shows the suppression of activity when manganese is added. The second panel in *B* was recorded at lower speed as indicated below that panel in *B*. All traces are from the same preparation.

DISCUSSION

The present results show that in Purkinje fibres the local anaesthetics benzocaine and procaine (1) decrease the action potential upstroke and duration, (2) depress markedly the contractile force, (3) antagonize strophanthidin induced spontaneous activity but not its positive inotropic effect, (4) reduce but do not block the positive inotropic effect of norepinephrine and Ca, and (5) do not cause Ca overload and in fact reduce it if present.

The mode of action of local anaesthetics certainly includes an impairment of the fast Na conductance which is activated during the upstroke. Since the demonstration of this effect in Purkinje fibres by Weidmann (1955), several investigators have reported similar findings in heart tissues (Carmeliet & Verdonck, 1974; Josephson & Sperelakis, 1976; Reiser, Freeman & Greenspan, 1974; Weld & Bigger, 1975), skeletal muscle (Bianchi & Shanes, 1959; Thesleff, 1956; Inoue & Frank, 1962), nerves (Shanes, Freygang, Grundfest & Amatniek, 1959; Taylor, 1959), and uterine muscle (Feinstein, 1966). By means of voltage clamp, a block of the fast inward Na current was clearly demonstrated (Hille, 1977). The reduction of the amplitude of the upstroke in the present experiments is in line with reported findings (see Fig. 5 in Weidmann, 1955).

The marked shortening ($\simeq -50\%$) of the action potential duration could also be

related to a curtailment of Na influx. The fast Na channel is known to inactivate within a few milliseconds. However, recent experiments provide evidence for a steady-state Na current in the potential range between -65 and -10 mV (Attwell, Cohen, Eisner, Ohba & Ojeda, 1979). Such current would be expected to be sensitive to local anaesthetics (as it is to tetrodotoxin, Attwell et al. 1979), in line with the fact that the local anaesthetic aprindine shifts the membrane potential of fibres depolarized in K-free solution to more negative values without increasing K efflux (Carmeliet & Verdonck, 1974). A curtailment of a background Na current by the local anaesthetics is also possible but appears less likely in view of the fall in the maximum diastolic potential and the slight steepening of diastolic depolarization with benzocaine.

The reduction in twitch force by local anaesthetics could be due to several causes. One possibility is that local anaesthetics decrease the slow inward current. In fact, local anaesthetics decrease Ca movements in several tissues (Shanes et al. 1959; Taylor, 1959; Feinstein, 1966) including cardiac tissues (Reiser et al. 1974; Josephson & Sperelakis, 1976). Furthermore, Eisner et al. (1979) have reported that tetracaine (5 mM) abolishes the slow inward current and tension development. However, several other investigators have failed to observe in cardiac tissues changes which could be ascribed to a depression of an inward Ca current (Kohlhardt, Bauer, Krause & Fleckenstein, 1972; Hagiwara & Nakajima, 1966; Carmeliet & Verdonck, 1974). The present results also argue against a block of the slow channel by procaine and benzocaine since the positive inotropic effect of norepinephrine (Fig. 4) and high Ca (Fig. 5) occurred also in the presence of local anaesthetics. Furthermore, procaine failed to abolish the slow reponse (in contrast to Mn, Fig. 10), in agreement with the results of Elharrar et al. (1977) and Brennan, Cranefield & Wit (1978). Some of the apparent discrepancies in the literature are likely to result from the different experimental conditions. For example, tetracaine abolished the slow inward current (Eisner et al. 1979) while procaine failed to abolish the slow response (Fig. 10). However, not only was the concentration of tetracaine 20 times larger than that of procaine but tetracaine is known to be more potent than procaine (Caputo et al. 1979). It is true that in the presence of local anaesthetics, norepinephrine and high Ca (in contrast to strophanthidin) increased force less. But this would be expected because only strophanthidin (by inhibiting the Na–K pump) would restore the intracellular Na concentration decreased by local anaesthetics (see below and Deitmer & Ellis, 1980).

That local anaesthetics decrease twitch force by blocking Ca re-uptake into the sarcoplasmic reticulum also appears unlikely for the following reasons: (1) the increase in force by caffeine was greater in the presence of high Ca and local anaesthetics (Fig. 7); (2) in the presence of high Ca and caffeine, procaine increased the force of contraction (Fig. 8); (3) the positive inotropic effect of strophanthidin (Figs. 2 and 3), norepinephrine (Fig. 4), high Ca (Fig. 5), caffeine (Fig. 6) and low Na (Fig. 9) persisted in the presence of local anaesthetics.

Another possibility is that local anaesthetics act by blocking the release of Ca from the sarcoplasmic reticulum as proposed for frog skeletal muscle fibres (Almers & Best, 1976; Caputo et al. 1979). Comparison of these with the present results is hindered by the use of different tissues, different agents, different potencies and different doses (the concentration of procaine used by Caputo et al. was 50–100 times larger than

the concentration used by us). If a block of Ca release occurs also in Purkinje fibres at the lower concentration used in the present experiments, the block should be only partial for the reasons discussed in relation to the possible block of Ca re-uptake.

In view of the present results, it is possible that both the plateau shift in a negative direction and the fall in twitch force are due to a curtailment of the inward movement of sodium by local anaesthetics. A decreased Na influx should result in a decreased $[Na]_i$ which in turn leads to a fall in intracellular Ca (Reuter & Seitz, 1968; Baker, Blaustein, Hodgkin & Steinhardt, 1969). That indeed local anaesthetics decrease the intracellular Na in the absence and presence of strophanthidin has been shown in a report (Deitmer & Ellis, 1980) which has appeared since the submission of the present manuscript. A decrease in intracellular Ca below normal level should result in a decrease in force of contraction and this decrease did occur in Tyrode solution (Fig. 1). In addition, the fall in twitch force was more pronounced when $[Na]_o$ was lower.

Eisner & Lederer (1979) report that in fibres exposed to low K, lidocaine decreases the transient inward current, the aftercontraction, the tonic tension and the twitch tension. In the presence of strophanthidin, instead, we find that procaine and benzocaine decrease the oscillatory potential in diastole while at the same time increase the magnitude of the twitch. The reason for this difference is not apparent (although the action of low K does not need to be necessarily identical to that of cardiac glycosides in every respect); but it should be pointed out that in the presence of strophanthidin, tetracaine (0·5 mM) decreases the aftercontraction and at the same time increases the twitch tension (see Fig. 9 in Tsien, Weingart & Kass, 1978) in agreement with our findings. The increase in force of contraction by local anaesthetics in the presence of strophanthidin (Fig. 2) and high Ca (Figs. 8 and 9) could still be explained by a fall in intracellular Ca (from an excessive toward an optimal level). It is generally agreed that toxic concentrations of cardiac glycosides increase intracellular Ca (see Lee & Klaus, 1971). This increase is brought about by the inhibition of the Na pump and the consequent accumulation of Na intracellularly. The 'Ca overload' thus brought about results in the oscillatory release of Ca from intracellular stores which is responsible for the induction of the oscillatory potentials and of the aftercontractions in diastole (Kass *et al.* 1978*a, b*). In the absence of cardiac glycosides, Ca overload can be brought about by increasing Ca or decreasing Na in the perfusing solution (see Kass *et al.* 1978*a* for references). Available evidence suggests that Ca overload results also in a decreased force of contraction whether overload is brought about by strophanthidin, high Ca, low Na or a combination thereof: when Ca overload is present, decreasing the extracellular Ca quickly leads to a transient increase in force (Vassalle & Lin, 1979). If local anaesthetics decrease intracellular Ca under normal conditions (and thereby decrease force), they should decrease the Ca overload when present. That procaine and benzocaine decrease Ca overload is supported by the following findings: (1) the transient oscillations are reduced (Fig. 2); (2) the twitch force increases; (3) caffeine increases force in the presence of high Ca and local anaesthetics while decreasing it in the presence of high Ca alone (Fig. 7); (4) procaine increases force in the presence of high Ca and caffeine while decreasing it in the presence of caffeine alone (Fig. 8); (5) local anaesthetics cause a rebound increase in force when the preparation is exposed to high Ca; (6) low

Na increases force in the presence of local anaesthetics and high Ca (Fig. 9); (7) tetracaine (0·5 mM) decreases the amplitude of the aftercontraction while increasing the amplitude of the twitch (see Fig. 9 of Tsien *et al.* 1978).

Transient oscillations are reduced by other anaesthetic agents (aprindine, Elharrar *et al.* 1977; lidocaine, Rosen & Danilo, 1980). In fact, licocaine reduces the transient inward current, the tonic force and the aftercontractions which are present in low K (Eisner & Lederer, 1979).

Since Na plays a major role in carrying the transient inward current responsible for the transient oscillatory potential (Kass *et al.* 1978*b*), local anaesthetics could counteract digitalis arrhythmias by blocking that current directly. The demonstration that local anaesthetics decrease the oscillatory potential while increasing the twitch force (present experiments) and decrease the aftercontraction (Tsien *et al.* 1978) suggests that a direct block of the transient inward current is unlikely to be the only mechanism. Rather, the findings are consistent with a decrease in Ca overload brought about by a decreased Na influx and intracellular Na concentration (Deitmer & Ellis, 1980), as proposed by Perry *et al.* (1978). In this connexion, it is interesting to note that tetrodotoxin which is known to decrease the inward movement of sodium (see Kao, 1966) but not that of Ca (Rougier, Vassort, Garnier, Gargouïl & Coraboeuf, 1969) also decreases the oscillatory potentials induced by cardiac glycosides in cardiac Purkinje fibres (Vassalle, 1975; Vassalle & Scida, 1979; Rosen & Danilo, 1980).

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